Estimation of Serum Urea
Objective:

• Estimation of Blood urea nitrogen (BUN) in serum sample.
-Urea:

- Urea is the highest non-protein nitrogen compound in the blood.

- Urea is the major excretory product of protein metabolism.

- Since historic assays for urea were based on measurement of nitrogen, the term blood urea nitrogen (BUN) has been used to refer to urea determination.
- **Urea synthesis:**

  - **Protein catabolism** produces amino acids that can be oxidized.
  
  - This results in the release of **ammonia** which is converted to **urea** (via **urea cycle in the liver**).
  
  - Following synthesis in the liver, urea is carried out in the **blood to the kidney** which is readily filtered from the plasma by **glomerulus**.
  
  - **Most of the urea** in the glomerular filtrate excreted in the urine, and **some urea is reabsorbed** through the renal tubules.
  
  - The amount reabsorbed **depends on urine flow rate and extent of hydration** (the amount of urea reabsorbed increases with dehydration).
-Urea synthesis:
- Clinical Application:

- Measurement of urea used in:
  - Evaluate renal function.
  - To assess hydration status.
  - To determine nitrogen balance.
  - To aid in the diagnosis of renal diseases.
  - Check a person's protein balance.
- **Plasma urea Concentration:**

  • Measurement of Blood Urea Nitrogen (BUN) alone is **less useful in diagnosing kidney diseases** because it’s blood level is influenced by **dietary protein and hepatic function.**

  • But its diagnostic value improves with **serum creatinine values.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High urea</strong></td>
<td><strong>Pre-renal</strong></td>
</tr>
<tr>
<td>(High urea conc. in plasma is called <strong>azotemia</strong>)</td>
<td>• Cognitive heart failure.</td>
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<tr>
<td></td>
<td>• Dehydration.</td>
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<tr>
<td></td>
<td>• High protein diet.</td>
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<td></td>
<td>• Increased protein catabolism.</td>
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<td></td>
<td><strong>Renal</strong></td>
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<td>• Renal failure.</td>
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<td><strong>Post-renal</strong></td>
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<td></td>
<td>• Urinary tract obstruction.</td>
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<td><strong>Low urea</strong></td>
<td><strong>Post-renal</strong></td>
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<tr>
<td></td>
<td>• Low protein intake.</td>
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<td></td>
<td>• Liver disease.</td>
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<td>• Pregnancy.</td>
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Caused by reduced renal blood flow, less blood is delivered to kidney, then less urea is filtered.

During pregnancy, The glomerular filtration rate increases by 50%
Practical Part
-Principle (of the kit used):

- The Reagent used contains: Urease, Glutamate Dehydrogenase (GLDH), NADH, α-ketoglutaric acid, buffers and stabilizers.

- This test involves two reactions:

1. \( \text{NH}_2 - \text{CO} - \text{NH}_2 + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2 \)

2. \( \alpha\text{-KETOGLUTARIC ACID} + \text{NADH} + \text{H}^+ \xrightarrow{\text{GLDH}} \text{GLUTAMIC ACID} + \text{NAD}^+ + \text{H}_2\text{O} \)

- The absorbance at 340nm is measured over a limited time period, resulting in decreased readings due to the oxidation of NADH to NAD.
-Method:

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Serum</th>
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<tbody>
<tr>
<td><strong>Reconstituted Reagent</strong></td>
<td>3ml</td>
<td>3ml</td>
</tr>
<tr>
<td><strong>Pre-warm at 37°C for 2 min. and add:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>0.025/25μl</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>0.025/25μl</td>
</tr>
</tbody>
</table>

- After exactly 30 seconds, read and record absorbance A1 against distilled water at 340 nm.
- At exactly another 60 seconds after A1, read and record the absorbance A2 and determine ΔA (A1-A2).
- Calculations:

- Concentration of urea in serum sample:

  - Standard concentration = 25 mg/dl

  - Urea (mg/dL) = \( \frac{\Delta A \text{ (Sample)}}{\Delta A \text{ (Standard)}} \times 25 \)

- Discussion:

  • Comment on the level of Urea in serum.
-Reference Value:

Blood urea conc.: (10 – 50 mg/ dl)
Estimation Of Arginase Activity In Liver Extract
- **Introduction:**

- Ammonia is a product of oxidative deamination of amino acids.

- It is toxic in even small amount and it must be removed from the body.

- Arginase is one of the important enzymes in urea cycle which is the major disposal form of amino groups derived from amino acids.

- Urea cycle catalyzed by a set of enzymes (Five enzymes) present in the liver, and then is transported in the blood to the kidneys for excretion.
Urea Cycle

1. Carbamoyl Phosphate Synthase

2. Ornithine carbamoyltransferase

3. Arginosuccinate synthase

4. Argininosuccinate lysase

5. Arginase

CO₂ + NH₃ → Carbamoyl Phosphate → Citrulline → L-Aspartate → Arginosuccinate → L-Fumarate → L-Arginine → Urea → H₂O
- **Principle:**

- The arginase enzyme catalyzes the **fifth reaction** in the urea cycle, the enzyme is present **exclusively in the liver**.

- Arginase catalyzes the hydrolytic cleavage of the guanidine group of Arginine to regenerate ornithine and urea.

  \[
  \text{Arginine} \leftrightarrow \text{Urea} + \text{Ornithine}
  \]

- Two isozymes of this Enzyme exist,

  - First; Arginase I (**In cytoplasm**) for functions of urea cycle,
  - Second; Arginase II to regulate the arginine/ornithine concentration in the cell (**In mitochondria**).

- Arginase requires a two-molecules metal of **Co}^{2+} and Mn}^{2+}** for its activation while **ornithine and lysine** are potent inhibitors.
-The activity of the enzyme is determined by **measuring the amount of urea produced**, urea is reacted with the reagent iso-nitrosopropiophenone and heated in boiling water, leading to the production of a red color compound which is measured by spectrophotometry at 520nm.

\[
\text{Urea + iso-nitrosopropiophenone} \xrightarrow{\text{boiling water bath}} \text{red color compound}
\]
- Question:

- What are the causes of high blood ammonia level?